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A further search for selective antagonists at M_2-muscarinic receptors

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1 In an attempt to obtain more selective antagonists acting at muscarinic M_2-receptors, analogues of 4-diphenylacetoxy-N-methylpiperidine methobromide (4-DAMP methobromide) have been synthesized. These were tested, along with silabenzhexol, procyclidine, sila-procyclidine and AFDX-116, in dose-ratio experiments with guinea-pig isolated atria at 30°C and ileum at 30°C and 37°C. The agonist was carbachol and the selectivity was assessed from the difference between log K for receptors in ileum and log K for receptors in atria.

2 The selectivity was not related to the affinity and some weakly active compounds retained appreciable selectivity but no compound had greater selectivity than 4-DAMP methobromide or pentamethylene bis-(4-diphenylacetoxy-N-methylpiperidinium) bromide.

3 Structure-activity relations are discussed. There seem to be steric limits to affinity but there are no obvious indications of the structural features associated with selectivity. It is suggested that more selective drugs may be obtained by introducing groups which may reduce affinity.

Introduction

Metho-salts of 4-diphenylacetoxy N-methylpiperidine (4-DAMP methiodide or methobromide) have greater affinity for muscarinic receptors in guinea-pig ileum than for those in guinea-pig atria (Barlow *et al.*, 1976). If two molecules are linked together with a pentamethylene chain a compound (bis-5 4-DAMP bromide) is obtained which is weaker but more selective (Barlow & Shepherd, 1985). In this paper the results obtained in a further search for compounds which differentiate between muscarinic receptors in guinea-pig ileum and guinea-pig atria are presented. It includes tests made with other compounds reported to be selective, such as sila-procyclidine (Mutschler & Lambrecht, 1984) and AFDX-116 (Hammer *et al.*, 1986; Giraldo *et al.*, 1986).

Methods

Guinea-pig isolated ileum

The guinea-pig ileum was set up as described by Edinburgh Staff (1974) with the responses recorded isotonically and a load of about 0.5 g. The agonist, carbachol, was allowed to act for 30 s and added once every 90 s by relays controlled from a PET microcomputer. The tissue was suspended in Krebs solution (Edinburgh Staff, 1974) aerated with a mixture of 95% O_2 and 5% CO_2, usually containing *nor*phenylephrine, 5 μM (see below), and, as in previous work (Barlow *et al.*, 1976), experiments were done at 29.8 ± 0.3°C and 37.0 ± 0.1°C. The lower temperature was needed for comparisons with results on atria and the effect of temperature on dose-ratio should give some indication of the enthalpy of binding of the antagonist.

Alternate small and large control responses were obtained, usually to 0.1 and 0.2 μM carbachol. When these were regular the tissue was exposed to a solution of the antagonist and the concentration of agonist was increased to try to obtain responses which roughly matched the controls. When these were regular the approximate dose-ratio is given by the ratio of the concentrations of agonist used in the presence and in the absence of the antagonist and an exact dose-ratio was calculated from the size of the responses by a calculation similar to a 4-point assay (Edinburgh Staff, 1974; Barlow, 1983). Usually a second concentration of antagonist was tested on each preparation and where possible a fresh set of control responses was obtained.

Guinea-pig isolated atria

The atria were set up in Krebs solution (Edinburgh Staff, 1974) aerated with a mixture of 95% O_2 and 5%

[1] Correspondence.

CO_2, usually containing *nor*phenylephrine, 5 μM (the same solution as was used for the ileum). The temperature was 29.8 ± 0.3°C and the spontaneous contractions were recorded isometrically with a load of about 0.2 g: action potentials were also recorded and the time required for 50 beats was continuously printed out (Barlow & Kitchen, 1982; Barlow & Shepherd, 1985).

The agonist, carbachol, was added by relays operated from a Commodore 128 microcomputer and allowed to act for 5 min: doses were given once every 15 min, with a second wash 10 min from the start of the cycle. The effects of the agonist were expressed as the percentage inhibition of the force of the contraction and the percentage increase in the time for 50 beats. As in the experiments on ileum, the control responses were usually obtained with 0.1 and 0.2 μM carbachol. The tissue was then exposed to the antagonist and the experiment continued as with the ileum.

These methods are similar to those previously described (Barlow *et al.*, 1976; Barlow & Kitchen, 1982) except that, as described by Barlow & Shepherd (1985), Krebs solution, aerated with a mixture of 95% O_2 and 5% CO_2, was used for all tissues. No hexamethonium was present but in this work the solution contained 5 μM *nor*phenylephrine, which prevented the gradual slowing down of the spontaneously beating atria during the experiment. This compound was chosen because it acts like noradrenaline and is chemically stable. The concentration did not affect the responses of the ileum to carbachol: there was no detectable change in the maximum, slope and EC_{50} of the log dose-response curve.

Its effects on dose-ratios were investigated by doing experiments with (±)-*p*-methyl 4-DAMP bromide in the presence and in the absence of 5 μM *nor*phenylephrine. The results are shown in Table 1. These were obtained as 4 separate sets consisting of measurements (with two concentrations of antagonist) on atria at 30°C, on ileum at 30°C and on ileum at 37°C. Two sets were made with Krebs solution containing *nor*phenylephrine and two sets were made without it. The dose-ratios do not appear to be affected by the presence or absence of 5 μM *nor*phenylephrine. The mean of all the values obtained in the presence of *nor*phenylephrine was 30.9, compared with 30.8 without it. When a Mann-Whitney U-test was made with the two groups, with and without *nor*phenylephrine, the values of U (122 and 134, with 16 in each group) exceeded the limits for a difference even with $P = 0.1$ (for which U should be less than 83).

Synthetic chemistry

All starting materials were obtained from Aldrich. The final products were recrystallized from combinations of ethanol, acetone, ethylmethyl ketone and ether. Their structures were checked by n.m.r. and they gave satisfactory micro-analyses. A table of melting-points and analyses is available from the authors.

For the preparation of 'reversed esters', *iso*nipecotic acid was methylated, using formaldehyde and formic acid, and converted into its chloride hydrochloride. This reacted with 9-hydroxyfluorene and the ester formed was isolated and methylated to give compound 18. However, this method did not work with diphenylmethanol so N-methyl-*iso*nipecotic acid was refluxed with benzhydryl bromide in xylene for a week. The ester was isolated and methylated to give compound 1. When benzhydryl bromide was refluxed in xylene with 4-hydroxy-N-methylpiperidine, an ether was formed much more quickly (within 24 h) and this was isolated and methylated to give compound 2.

Chloro-diphenylacetyl chloride and bromo-diphenylacetyl bromide were esterified with 4-hydroxy-N-methyl piperidine and the bases extracted and meth-

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Table 1 Dose-ratios for (±)-mono-*p*-methyl-4-diphenylacetoxy-N-methylpiperidine methobromide with (*) and without *nor*phenylephrine

	Ileum			
	30°C		*37°C*	
0.1 μM				
	14.6	14.8*	14.0	13.1*
	15.9	18.6*	18.1	16.1*
0.5 μM				
	74.2	53.6*	66.5	69.1*
	57.8	88.3*	80.7	74.7*
	Atria			
	Rate		*Force*	
0.1 μM				
	4.53	7.61*	3.66	4.80*
	8.38	11.2*	9.01	8.20*
0.5 μM				
	23.4	24.5*	14.4	22.7*
	43.5	34.0*	44.1	33.4*

ylated, to give compounds 10 and 11. The bromine is labile and when the bromo-ester is allowed to stand in methanol the methoxy ester is obtained which was methylated to give compound 9.

Diphenylpropionic acid, diphenyl-α-methylacetic acid, *cyclo*hexylphenyl acetic acid, anthracene-9-carboxylic acid, adamantane-1-carboxylic acid and triphenylacetic acid were converted to their acid chlorides, and then esterified with 4-hydroxy-N-methylpiperidine and the bases methylated, to give compounds 3, 8, 16, 19, 23 and 25. Anthracene-9-carboxylic acid was reduced to the dihydro-compound with sodium and alcohol and this was converted to the acid chloride, esterified and methylated to give compound 20. Di-(2-thiophenyl)acetic acid was prepared from the reaction of thiophene (2 mol) with chloral hydrate in concentrated H_2SO_4 (Freeman *et al.*, 1948) and hydrolysing the 1,1-dithiophenyl-3,3,3-trichloroethane formed to obtain the substituted acetic acid. This was converted into compound 17 via the acid chloride.

Compound 4 was prepared from diphenylacetyl chloride and 4-hydroxy tetrahydro-thiopyrane, obtained from reduction of tetrahydro thiopyran-4-one with $NaBH_4$. The ester reacted readily with methyl iodide to give the sulphonium compound.

The ethyl and *n*-propyl homologues of 4-DAMP, compounds 5 and 6, were obtained by treating the base, 4-diphenylacetoxy-N-methylpiperidine with ethyl- and *n*-propyl- bromides, respectively. The hydroxyethyl derivative, compound 7, was obtained from the secondary amine, 4-diphenylacetoxy piperidine, which was prepared by catalytic debenzylation of the N-benzyl compound. The secondary amine was treated with ethylene bromhydrin and then quaternized with methyl bromide.

The *o*-, *m*-, and *p*-methyl- and *p*-phenyl compounds (12, 13, 14 and 24) were obtained from the corresponding benzophenones which were treated with sodium and carbon dioxide (Hamrick & Hauser, 1959) to give the diphenyl-glycollic acid and then with red phosphorus and HI to reduce out the hydroxyl group and give the substituted phenylacetic acid. This was converted into the acid chloride, esterified and methylated. In a similar way dibenzosuberone was converted into the dibenzo-*cyclo*heptane carboxylic acid and on to give compound 22. With thioxanthene-9-one, however, the method of Heacock *et al.* (1958) was used: the keto group was reduced to $-CH_2-$ with sodium and alcohol and this was treated with butyllithium followed by carbon dioxide. The acid was then converted into the acid chloride, esterified and methylated to obtain compound 21.

The di-*o*-chloro compound (15) was obtained by converting *o*-chlorobenzaldehyde to the benzoin, benzil, benzilic acid and phenylacetic acid (cf. the preparation of the di-*p*-tolyl compound, Barlow & Shepherd, 1985).

The diphenylacetyl amide (compound 26) was the sample described by Barlow *et al.* (1978).

Compounds

Carbachol chloride was obtained from Sigma and *nor*phenylephrine hydrochloride from Aldrich. The following compounds were gifts for which we are most grateful: (±)-sila-benzhexol from Dr R.M. Eglen, Syntex Research Centre, Edinburgh; (±)-, (−)- and (±) sila-procyclidine from Dr G. Lambrecht, Department of Pharmacology, Frankfurt and AFDX-116 (11-[[2-[(diethylamino)methyl]-1-piperidinyl]acetyl]-5, 11-dihydro-6H-pyrido[2,3-b][1,4]benzodiazepin-6-on) from Boehringer Ingelheim.

Results

The results are summarized in Table 2. The dose-ratios for each concentration have been used to calculate the affinity constant and the mean estimate of log affinity constant (K) is shown (± s.e.). The asterisk indicates experiments done in the presence of *nor*phenylephrine and the two asterisks indicate the *p*-methyl compound tested with and without *nor*phenylephrine (for which the dose-ratios are given in Table 1). If the compound behaves competitively the mean estimate of log K should be the same at all concentrations: this seems to be true for the range of concentrations studied. Likewise there are no obvious differences between results obtained using effects on atrial rate and those obtained using effects on atrial force.

The overall mean values for effects of all concentrations on the ileum at 30°C and for all concentrations on atrial rate and atrial force have been used to prepare Figure 1, in which the compounds are indicated by their number in Table 2 and the horizontal line indicates the selectivity, being the difference between log K for ileum and log K for atria. (For compounds 5 and 6 log K for ileum at 37°C was used because measurements were not made on ileum at 30°C.) The compounds X1 to X6 are included for comparison: X1 is 4-diphenylacetoxy-N-methylpiperidine hydrochloride (4-DAMP not quaternized) and X2 is the benzilic ester of 4-hydroxy-N-methylpiperidine methiodide (with hydroxyl in place of hydrogen attached to the acetyl group). The results for these compounds are taken from Barlow *et al.* (1976). The results for the other compounds are given by Barlow & Shepherd (1985): X3 is *p*-dimethyl-4-DAMP methiodide, X4 is the *p*-dichloro-compound: X5 and X6 are, respectively, the fluorene-9-carboxy- and xanthene-9-carboxy- analogues.

Note that with all the compounds except AFDX-116 (shown as a broken line) the affinity for the ileum is greater than that for the atria.

Table 2 Log affinity constants

Conc (μM)	Atria		Ileum	
	Rate	Force	30°C	37°C
I* (±)-Sila-benzhexol				
0.1	7.64 ± 0.04 (4)	7.60 ± 0.12 (4)	8.24 ± 0.05 (4)	8.19 ± 0.04 (4)
0.5	7.66 ± 0.08 (4)	7.50 ± 0.07 (4)	8.19 ± 0.08 (4)	8.20 ± 0.04 (4)
II* (±)-Procyclidine				
0.5	7.08 ± 0.09 (5)	7.06 ± 0.06 (5)	7.77 ± 0.03 (6)	7.78 ± 0.02 (6)
2.0	7.10 ± 0.10 (5)	7.09 ± 0.05 (5)	7.74 ± 0.05 (5)	7.78 ± 0.02 (5)
III* (−)-Silaprocyclidine				
0.1	7.43 ± 0.26 (3)	7.52 ± 0.07 (3)	8.43 ± 0.06 (3)	8.39 ± 0.03 (3)
IV* (+)-Silaprocyclidine				
0.1	7.56 ± 0.06 (3)	7.46 ± 0.04 (3)	8.31 ± 0.07 (3)	8.07 ± 0.06 (3)
V* AFDX-116				
1.0	7.26 ± 0.14 (2)	7.27 ± 0.04 (2)	6.15 ± 0.03 (2)	6.14 ± 0.12 (2)
2.0	7.36 ± 0.14 (3)	7.31 ± 0.05 (3)	6.22 ± 0.06 (3)	6.12 ± 0.04 (3)
10.0	7.35 ± 0.14 (3)	7.27 ± 0.09 (3)	6.16 ± 0.04 (3)	6.06 ± 0.08 (3)
1 Reversed 4-DAMP MeBr (-O-CO- replaced by -CO-O-)				
0.1	7.06 ± 0.07 (3)	7.24 ± 0.16 (3)	7.81 ± 0.13 (3)	7.85 ± 0.15 (3)
0.5	7.26 ± 0.08 (3)	7.35 ± 0.12 (3)	7.77 ± 0.17 (3)	7.85 ± 0.15 (3)
2 4-Diphenylmethoxy-N-methylpiperidine MeBr (short 4-DAMP ether)				
0.1	7.60 ± 0.12 (3)	7.69 ± 0.09 (3)	8.01 ± 0.04 (4)	8.02 ± 0.07 (4)
0.5	7.78 ± 0.12 (3)	7.79 ± 0.08 (3)	7.98 ± 0.06 (4)	8.03 ± 0.04 (4)
3 4-Diphenylpropionyl-N-methylpiperidine MeBr (homo-4-DAMP)				
5.0	5.61 ± 0.23 (4)	5.74 ± 0.12 (4)	6.26 ± 0.03 (4)	6.20 ± 0.12 (4)
10.0	5.65 ± 0.19 (4)	5.71 ± 0.09 (4)	6.23 ± 0.04 (5)	6.21 ± 0.10 (5)
50.0	5.61 ± 0.06 (3)	5.61 ± 0.07 (3)	6.21 ± 0.02 (4)	6.13 ± 0.09 (4)
4* Sulphonium analogue of 4-DAMP MeBr				
0.1	7.42 ± 0.18 (4)	7.48 ± 0.06 (4)	7.86 ± 0.05 (4)	7.78 ± 0.03 (4)
0.5	7.35 ± 0.13 (4)	7.48 ± 0.03 (4)	7.88 ± 0.04 (4)	7.82 ± 0.03 (4)
5 4-Diphenylacetoxy-N-methylpiperidine EtBr				
0.1	7.82 ± 0.13 (4)			
0.5	7.70 ± 0.05 (3)			8.67 ± 0.05 (4)
2.0				8.65 ± 0.02 (4)
6 4-Diphenylacetoxy-N-methylpiperidine *n*-PrBr				8.70 ± 0.03 (4)
0.5	6.49 ± 0.10 (4)			
2.0				7.41 ± 0.05 (4)
7 4-Diphenylacetoxy-N-methylpiperidine-hydroxyethyl bromide				7.35 ± 0.02 (4)
0.1	7.43 ± 0.06 (3)	7.20 ± 0.03 (4)	7.88 ± 0.03 (3)	7.91 ± 0.01 (4)
0.5	7.38 ± 0.05 (3)	7.31 ± 0.03 (4)	7.79 ± 0.05 (3)	7.91 ± 0.04 (4)
8* 4-Diphenyl-methyl-acetoxy-N-methylpiperidine MeBr (α-methyl 4-DAMP)				
0.01	9.25 ± 0.08 (4)	9.22 ± 0.09 (4)	9.68 ± 0.03 (4)	9.50 ± 0.08 (4)
0.02	9.33 ± 0.13 (2)	9.46 ± 0.13 (2)	9.62 ± 0.01 (2)	9.70 ± 0.21 (2)
0.05	9.32 ± 0.04 (3)	9.28 ± 0.06 (3)	9.77 ± 0.05 (3)	9.57 ± 0.06 (3)
0.1	9.26 ± 0.06 (3)	9.40 ± 0.10 (3)	9.64 ± 0.02 (3)	9.62 ± 0.09 (3)
9* 4-Diphenyl-methoxy-acetoxy-N-methylpiperidine MeBr (α-methoxy 4-DAMP)				
0.1	7.77 ± 0.09 (4)	7.62 ± 0.09 (4)	8.20 ± 0.02 (4)	8.16 ± 0.03 (4)
0.5	8.02 ± 0.10 (3)	8.02 ± 0.12 (3)	8.28 ± 0.04 (4)	8.25 ± 0.01 (4)
10 4-Diphenyl-chloro-acetoxy-N-methylpiperidine MeBr (α-chloro 4-DAMP)				
0.02	9.48 ± 0.10 (4)	9.42 ± 0.04 (4)	9.39 ± 0.11 (4)	9.31 ± 0.05 (4)
0.1	9.47 ± 0.11 (4)	9.38 ± 0.05 (4)	9.54 ± 0.25 (4)	9.69 ± 0.06 (4)
11 4-Diphenyl-bromo-acetoxy-N-methylpiperidine MeBr (α-bromo 4-DAMP)				
0.02	9.26 ± 0.11 (4)	9.20 ± 0.17 (4)	9.30 ± 0.02 (6)	9.32 ± 0.04 (6)
0.1	9.26 ± 0.07 (4)	9.21 ± 0.06 (4)	9.51 ± 0.07 (6)	9.45 ± 0.04 (6)
12* (±)-Mono-o-methyl-4-DAMP MeBr				
0.1	7.25 ± 0.14 (2)	7.30 ± 0.06 (3)	7.46 ± 0.13 (3)	7.50 ± 0.09 (3)
0.5	7.24 ± 0.15 (2)	7.28 ± 0.01 (3)	7.42 ± 0.10 (3)	7.47 ± 0.10 (3)
13* (±)-Mono-m-methyl-4-DAMP MeBr				
0.1	7.40 ± 0.11 (5)	7.57 ± 0.06 (5)	8.07 ± 0.04 (5)	7.99 ± 0.03 (5)
0.5	7.44 ± 0.08 (5)	7.58 ± 0.06 (5)	8.07 ± 0.04 (5)	7.96 ± 0.03 (5)

Table 2 Log affinity constants

Conc (μM)	Atria		Ileum	
	Rate	Force	30°C	37°C
14** (±)-Mono-*p*-methyl-4-DAMP MeBr				
0.1	7.81 ± 0.10 (4)	7.69 ± 0.11 (4)	8.17 ± 0.03 (4)	8.15 ± 0.03 (4)
0.5	7.77 ± 0.07 (4)	7.70 ± 0.11 (4)	8.12 ± 0.05 (4)	8.16 ± 0.02 (4)
15* *o*-Dichloro-4-DAMP MeBr				
0.1	7.44 ± 0.08 (4)	7.36 ± 0.07 (4)	7.74 ± 0.02 (4)	7.68 ± 0.01 (4)
0.5	7.39 ± 0.02 (4)	7.40 ± 0.03 (4)	7.66 ± 0.03 (4)	7.67 ± 0.02 (4)
16 Phenyl-*cyclo*hexylacetoxy-N-methylpiperidine MeI (*cyclo*hexyl analogue)				
0.1	8.61 ± 0.01 (3)	8.50 ± 0.05 (3)	9.07 ± 0.09 (3)	9.36 ± 0.05 (3)
0.5	8.50 ± 0.03 (3)	8.42 ± 0.03 (3)	9.06 ± 0.07 (3)	9.33 ± 0.01 (3)
17 4-Di-2-thiophenylacetoxy-N-methylpiperidine MeBr				
0.1	8.97 ± 0.07 (4)	8.92 ± 0.05 (5)	9.32 ± 0.07 (5)	9.31 ± 0.03 (5)
0.25	9.03 ± 0.04 (3)	9.13 ± 0.13 (3)	9.16 ± 0.03 (5)	9.22 ± 0.04 (5)
18 9-Hydroxyfluorene ester of N-methyl-*iso*-nipecotic acid MeBr (reversed ester -O-CO- replaced by -CO-O-)				
0.1			7.35 ± 0.17 (4)	7.22 ± 0.09 (4)
0.5	7.03 ± 0.08 (3)	6.81 ± 0.08 (3)	7.29 ± 0.15 (4)	7.05 ± 0.11 (3)
19 4-(Anthracene-9-carboxy)-N-methylpiperidine MeBr				
2.0	6.67 ± 0.11 (4)	6.61 ± 0.08 (4)	6.87 ± 0.08 (4)	6.87 ± 0.08 (6)
10.0	6.67 ± 0.13 (3)	6.44 ± 0.05 (2)	6.93 ± 0.09 (4)	6.89 ± 0.08 (5)
20 4-(9,10-Dihydroanthracene-9-carboxy)-N-methylpiperidine MeBr				
0.1	8.04 ± 0.06 (4)	7.98 ± 0.05 (4)	8.51 ± 0.06 (4)	8.44 ± 0.08 (4)
0.5	7.89 ± 0.10 (3)	7.69 ± 0.06 (2)	8.45 ± 0.08 (4)	8.46 ± 0.10 (4)
21* 4-(Thioxanthen-9-carboxy)-N-methylpiperidine MeBr				
0.1	7.87 ± 0.14 (4)	7.74 ± 0.10 (4)	7.81 ± 0.02 (4)	7.81 ± 0.04 (4)
0.5	7.66 ± 0.06 (3)	7.66 ± 0.05 (3)	7.74 ± 0.04 (3)	7.70 ± 0.01 (3)
22* 4-(Dibenz*cyclo*heptane-carboxy)-N-methylpiperidine MeBr (ethane-bridged 4-DAMP)				
0.5	6.12 ± 0.17 (3)	5.93 ± 0.42 (5)	6.27 ± 0.02 (5)	6.35 ± 0.05 (4)
5.0	6.16 ± 0.01 (2)	6.13 ± 0.06 (4)	6.24 ± 0.02 (4)	6.34 ± 0.04 (4)
23* 4-(Adamantane-1-carboxy)-N-methylpiperidine MeBr				
1.0	5.85 ± 0.08 (4)	5.94 ± 0.04 (4)	6.65 ± 0.05 (4)	6.58 ± 0.08 (4)
5.0	5.88 ± 0.08 (4)	5.92 ± 0.04 (4)	6.62 ± 0.04 (4)	6.61 ± 0.06 (4)
24* (±)-Mono-*p*-phenyl 4-DAMP MeBr				
1.0	5.94 ± 0.06 (3)	5.87 ± 0.11 (3)	6.28 ± 0.03 (4)	6.08 ± 0.08 (4)
5.0	6.02 ± 0.05 (3)	5.81 ± 0.02 (3)	6.26 ± 0.04 (4)	6.15 ± 0.04 (4)
25 4-Triphenylacetoxy-N-methylpiperidine MeBr				
5.0	5.60 ± 0.13 (3)	5.71 ± 0.04 (3)	6.41 ± 0.04 (3)	6.36 ± 0.01 (3)
26 2-Diphenylacetamido-N-ethyldimethylamine ethiodide†				

*Indicates experiments done in the presence of *nor*phenylephrine, 5 μM. **Compound tested with and without *nor*phenylephrine and the results are shown in Table 1.
† Tested up to 10 μM: dose-ratio on ileum and atria about 5: log $K < 5.6$.

Discussion

In this work there appear to be several compounds which have greater affinity for muscarinic receptors in ileum than in atria but AFDX-116 clearly has greater affinity for receptors in atria than in ileum, so selectivity cannot be an artifact of the way the tests are made. There is considerable uncertainty about the degree of selectivity (Barlow & Shepherd, 1985) and it is perhaps not surprising that estimates of selectivity vary from one study to another. Our results with sila-benzhexol and sila-procyclidine indicate less selectivity than has been found in other work but it is clear, for instance, that sila-procyclidine (III) has greater activity and selectivity than procyclidine (II).

Selectivity does not appear to be associated with any particular degree of affinity: there are potent compounds with low selectivity and weak compounds with appreciable selectivity. The distribution of selectivity in Figure 1 can be explained by supposing that the receptors in atria and ileum are not identical. Unless a simpler explanation can be found it seems reasonable to seek to interpet the results by looking at the structures of the compounds to try to see if these suggest differences between the receptors with which the compounds interact.

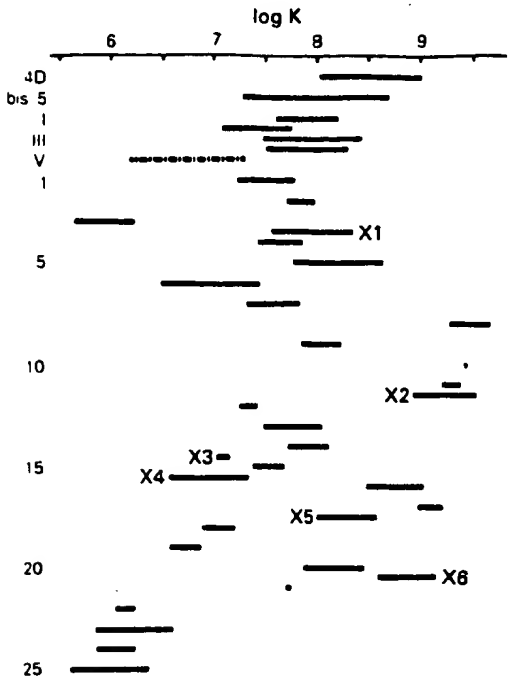

Figure 1 The log affinity constant (K: at 30°C in Krebs solution with carbachol as agonist) is shown for the compounds, numbered according to the list in Table 2. The compound at the top is 4-DAMP MeBr (4D) and results for pentamethylene *bis*-4-DAMP Br (bis-5) are shown below. The line indicates the selectivity: it is the difference between log K for the ileum and log K for the atria. With all the compounds except AFDX-116 (compound V, broken line) the value for the ileum is greater than that for the atria. Results for the compounds X1 to X6 are included for comparison: X1 is the tertiary compound (4-DAMP HCl), X2 is the benzilic ester of 4-hydroxy-N-methyl piperidine methiodide: X3 is di-*p*-methyl-4-DAMP MeBr: X4 is di-*p*-chloro- 4-DAMP MeBr: X5 and X6 are the fluorene-9-carboxy-and xanthene-9-carboxy- esters, respectively.

The general aim (limited by what it is possible to make) has been to investigate the effects of changing the ester group and chain length (compounds 1–3), changing the onium group (compounds 4–7), changing the α-hydrogen (compounds 8–11), substitution of the benzene ring (compounds 12–15 and 24), replacing a benzene ring (compounds 16 and 17) and linking the benzene rings together (compounds 18–23).

The extra methylene group in homo-4-DAMP (3) drastically reduces affinity but the compound retains some selectivity. Replacement of quaternary ammonium by tertiary sulphonium (4) reduces affinity and selectivity. If the compound is compared with 4-DAMP HCl (X1) it seems as if the loss of a methyl group has reduced affinity but the change of the atom from nitrogen to sulphur has reduced selectivity. Alkylation of the nitrogen also reduces affinity but not as drastically (5–7). Affinity is appreciably increased by α-methyl-, chloro- or bromo- groups (8, 10 and 11) but reduced by α-methoxy (9). This is surprising because the compounds are at least as active as the α-hydroxy compounds (the benzilic ester, X1) and it is usually assumed that the high affinity of hydroxy compounds can be ascribed to hydrogen bonding with the receptor, which cannot be true for methyl-, chloro- or bromo- compounds. A comparision of the crystal structures, however, shows that when the piperidine rings are superimposed, the hydroxyl group in the tropic acid part of (−)-hyoscine methiodide is in a very different position from the hydrogen on the acetyl part of 4-DAMP methiodide (Barlow, Howard, Johnson and Sanders, unpublished).

Either chloro- or methyl- substituents in the benzene ring reduce activity. The series of compounds 12, 13 and 14 suggests that a methyl group disturbs binding most in the *o*-position (12) and least in the *p*-position (14), but even in this position the compound is not as active or as selective as the unsubstituted 4-DAMP methobromide. The di-*p*-methyl- compound (X2) is even weaker than the mono-*o*-methyl compound (12). In contrast the di-*o*-chloro- compound (15) is more active that the di-*p*-chloro- compound (X3), which retains some selectivity, but both are much weaker than 4-DAMP methobromide.

Replacement of one benzene ring by cyclohexyl increases affinity (16) but reduces selectivity and replacement of two benzene rings by thiophene (17) increases affinity and reduces selectivity even further.

The reversed ester of fluorene (18) should be compared with the fluorene ester (X4) and both should be compared with 4-DAMP methobromide and its reversed ester (1). Reversing the ester group reduces affinity and selectivity more with the fluorene compounds, even though these are weaker. The anthracene compound (19) is remarkably weak but reduction to the 9,10-dihydro-compound (20) increases affinity almost 100 fold. The thioxanthene ester (21) should be compared with its oxygen analogue (X5) and the dihydro-anthracene compound (20) should be compared with the *cyclo*heptane analogue (22). These results all reinforce the idea that there are steric constraints to the binding of this part of the molecule. If it is very flat (19) or bulky (22) it cannot bind well. It is therefore not surprising that the adamantyl ester (23), *p*-phenyl 4-DAMP MeBr (24) and the triphenylacetic ester (25) are weak.

It is not clear, however, the extent to which electronic effects are involved in binding nor what combination of steric and electronic effects determine selectivity. It seems possible that, at this stage.

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progress in the development of selective compounds may be made by making changes likely to reduce affinity, in the hope that the reduction will be greater at one type of receptor than the other. This has not worked with the amide (26) but it is notable that the compound AFDX-116, which is undoubtedly selective, is also remarkably weak compared with atropine.

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